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Soil invertebrates infected by microorganisms

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Résumé : Étude sur les microorganismes pathogènes de la faune du sol

Les données sur les microorganismes pathogènes et leur rôle au sein de la faune du sol sont restées jusqu'ici très limitées.

De 1976 à 1981, des études ont été faites sur les maladies naturelles de la faune du sol dans différentes forêts de l'Europe. Deux localités où d'importantes émissions de SO_2 ont été constatées (Silberbron et Siegerland, Allemagne Fédérale), quatre localités en Espagne (Guernica, Munguia, Altube et Orduna, Pays Basque) et une en Autriche (Weissensee, Kaernten) ont été prises en considération dans nos recherches. La faune du sol, Nématodes, Enchytraéides, Lumbricides, Oribates, Collemboles, larves de Diptères et d'autres groupes d'Invertébrés furent récoltés dans des forêts de conifères, des forêts de feuillus, des forêts de hêtres et des forêts d'*Eucalyptus*.

L'étude sur les parasites responsables des maladies de la faune du sol faite à l'aide des microscopes photonique et électronique a montré la présence de quatre groupes de microorganismes : Protozoaires, Bactéries, Champignons et la présence d'un groupe d'organismes ressemblant à des virus : parmi ces parasites les Protozoaires étaient les plus abondants. La plupart de ces parasites se développent dans différents tissus des Invertébrés du sol et provoquent leur mortalité.

Nos analyses révèlent de très hauts pourcentages d'infections : 70 % chez les Enchytraéides, 45 % chez les Oribates, 25 % chez les Collemboles etc. dans les localités de Sillberborn et Siegerland (Allemagne Fédérale). Les Invertébrés du sol dans les régions où les émissions de SO_2 sont importantes sont plus affectés par les maladies tandis que, dans les régions plus naturelles (Pays Basque et Kaernten), la faune du sol était presque entièrement saine.

Key words : soil invertebrates, pathogenic agents, natural infections.

I. Introduction

Invertebrates and the whole system of organisms inhabiting soil have been studied by different authors. The attention of soil ecologists and biologists has been concentrated on the effects of environmental factors and human activites. However, the role of diseases in population dynamics of soil invertebrates in agrobiocenoses and forests has not been adequately investigated. Our investigations of the pathology of forest soil animals made during 1976-1981 revealed that various microorganisms close to virus-like organisms, bacteria, fungi, and protozoa are associated with soil invertebrates (PURRINI, 1980). The purpose of this study is to summarize the results of research on the incidence and distribution of different groups of microorganisms parasitizing soil animals. Sampling was performed at localities with seemingly different environmental properties. Possible influence of environmental factors are discussed.

II. Material and methods

A large number of forest mulch (litter) and soil samples were collected separately for litter and soil; one sample of litter consisted of 10 l collected from 10 x 10 m squares, and one sample of soil consisted of 1 l (5-10 cm) of soil taken with a steel frame. The litter and soil samples were treated in Tullgren funnels for 4-7 days, and separately for nematodes and enchytraeids in O'connor's wet funnels for one hour (1h). Samples containing nematodes, enchytraeids, earthworms, and arthropods (oribatid mites, springtails, dipterous larvae, and others) were collected in vials with water for further examinations. Thousands of specimens (Tables I and II) of soil animals were examined for parasites by means of light and electron microscopy. For diagnosis of infection, smear preparations were examined either unstained or fixed with methanol stained with Giemsa's stain. Paraffin sections, prepared after fixation in Bouin's fluid and stained with Heidenhain's iron haematoxylin, served for histological analysis. For electron microscopy, the whole body of infected oribatid mites or springtails, or parts of infected tissues of other animals were prepared in the usual way for embedding in Spurr's low viscosity or araldite resins and cut in ultrathin sections for examination with Philips 301 electron microscope.

TABLE I

The percent occurrence of different groups of parasites found in forest soil invertebrates during investigation period (1976-1981) in Federal Republic of Germany.

Soil invertebrates	Total of examined specimens (approx. numbers)	PARASITES (%)							
		Virus-like organisms	Bacteria	Fungi	Protozoa				
					Ciliata	Amoebida	Gregarinida	Coccidia	Microsporida
Nematoda	1000	0	0	0	0	0	10	10	0
Enchytraeidae	2600	0	4	5	5	0	50	0	6
Lumbricidae	1800	0	0	0	0	0	20	0	0
Oribatida	5500	3	5	5	0	2	20	5	10
Collembola	4500	2	0	2	0	0	2	6	9
Diptera	800	0	0	2	0	0	2	0	6
Other invertebrates	2000	2	0	2	0	0	10	0	4

TABLE II

Quantitative and qualitative survey of infected forest soil invertebrates in German Federal Republic (Silberborn and Siegerland : both localities with high loads of SO_2 -immissions) compared to localities examined in Spain and Austria.

Soil invertebrates	German Federal Republic				Spain								Austria	
	Silberborn		Siegerland		Guernica		Munguia		Altube		Orduna		Weissensee	
	Exam. N.	Inf. %	Exam. N.	Inf. %	Exam. N.	Inf. %	Exam. N.	Inf. %	Exam. N.	Inf. %	Exam. N.	Inf. %	Exam. N.	Inf. %
Nematoda	80	30	150	40	0	0	0	0	0	0	0	0	0	0
Enchytraeidae	300	60	200	80	25	10	30	5	60	20	100	10	250	10
Lumbricidae	200	30	100	20	10	0	20	0	20	0	50	0	50	0
Oribatida	800	40	900	50	80	2	100	2	150	0	200	0	300	2
Collembola	600	30	700	20	70	0	100	0	200	0	180	0	200	0
Diptera	200	10	100	5	30	0	30	0	50	0	0	0	30	0
Other invertebrates	500	10	200	20	10	0	50	0	10	0	20	0	50	0

Exam. = examined, Inf. = infected, N. = Number of examined specimens.

III. Results and discussion

Soil animals were collected in forests of different kinds in the German Federal Republic, Spain and Austria (Tables I and II). The subject of this study was two localities in Germany (Silberborn), Lower Saxony and Siegerland, Westphalia where high loads of SO_2 -immissions were observed, four in Spain (Viscaya : Guernica, Munguia, Altube and Orduna), and one in Austria (Kaernten Weissensee). Four types of forests : mixed coniferous (Germany and Spain), mixed deciduous (Germany, Spain and Austria), beech (Germany and Spain), and *Eucalyptus* forests (Spain) were examined. The localities in Spain and Austria where insecticides and herbicides have never been used, and situated far from main roads and industrial air pollution areas, seem to be more undisturbed.

A large number of specimens of soil invertebrates belonging to different groups of organisms were examined for parasites by means of light and electron microscopy. Four groups, enchytraeids, earthworms, oribatid mites and springtails, collectively named « decomposer organisms », were intensively studied because of their common occurrence in forest litter and soil samples and because their intensive transformation of organic material in forest soil plays an important role in nature. Three abundant species of enchytraeids, three of earthworms, 20 of oribatid mites and 12 of springtails in Germany were found to be infected by different microorganisms (Table I), most of them causing lethal diseases in their hosts. The soil fauna at localities examined in Spain and Austria was almost healthy except for some gregarines living as commensals in the gut lumen of host-animals. A survey of the ascertained infection rates in Spain and Austria compared to localities in Germany is given in Table II. The high infection rate in all groups of soil animals at localities with high loads of SO_2 -immissions (Silberborn and Siegerland, German F.R.) are shown in Table II : 70 % of enchytraeids, 45 % of oribatid mites and 25 % of springtails were infected.

Many important parasites such as *Helicosporidium parasiticum* Keilin 1921 (fungi), and other species close to five groups of Protozoa, *i.e.* Ciliata, Amoebida, Gregarinida, Coccidia and Microsporidia were found to be associated with « decomposer organisms » and other invertebrates (Table I and III). Generally, the enchytraeids (60 % in Silberborn and 80 % in Siegerland)., *Achipteria coleoptrata* (90 %), *Rhysotritia duplicata* (55 %) and *Platynothrus peltifer* (50 %) (Oribatida), *Onychiurus quadriocellatus* (55 %) and *Isotoma violacea* (30 %) (Collembola) show the highest infection rates at localities with high loads of SO_2 -immissions during the investigation period. However, the infection rate may be higher because the extraction method for soil animals revealed only motile specimens. It is likely that heavily infected animals remaining in the litter escaped examination. If the infected specimens were not as active as healthy ones they would have been unable to pass through the funnels and move from the litter to the sampling glasses with water.

TABLE III

A survey of infection rates in enchytraeids, lumbricids and some common species of oribatid mites and springtails in Silberborn and Siegerland (German Federal Republic).

		PARASITES (%)					
Soil invertebrates	**Total infected (%)**	**Fungi**	**Protozoa**				
		Helicosporidium parasiticum	**Ciliata**	**Amoebida**	**Gregarinida**	**Coccidia**	**Microsporida**
Enchytraeidae	70	2	10	0	50	0	8
Lumbricidae	25	0	0	0	25	0	0
Oribatida (Acarina):							
Achipteria coleoptrata	90	80	0	0	0	8	2
Rhysotritia duplicata	55	0	0	0	25	0	30
Nothrus silvestris	30	2	0	0	8	20	0
Platynothrus peltifer	50	20	0	0	10	15	5
Phthiracarus globosus	30	10	0	2	5	10	3
Phthiracarus piger	30	5	0	3	10	0	5
Cepheus dentatus	20	0	0	5	10	0	5
Collembola (Apterygota):							
Onychiurus quadriocellatus	55	0	0	0	0	0	55
Isotoma violacea	30	5	0	0	0	10	15
Folsomia quadrioculata	20	10	0	0	0	5	5
Lepidocyrtus lignorum	20	0	0	0	5	0	15

Our work raises certain important questions which need immediate attention, as very few papers (HESSE, 1909; COGNETTI DE MARTIIS, 1911; BERLIN, 1924; BHATIA, 1929; THOR, 1930; TROISI, 1933; DOLFFUS, 1946; MEIER, 1956; and others) have been published on the pathology of soil invertebrates. Our investigations (1976-1981) revealed some new groups of microorganisms, such as Ciliata, Amoebida, Coccidia and Microsporidia parasitizing soil animals, and new host records for *Helicosporidium parasticum* (fungi). Up to now, 26 news spcies, some amoeba-like organisms living in the gut lumen of oribatid mites (PURRINI, 1981), seven gregarines in oribatid mites and dipterous larvae (PURRINI *et al.*, 1979; PURRINI & ORMIERES, 1981), two coccidian parasites (PURRINI, 1981) and microsporidians in oribatid mites (PURRINI & WEISER, 1981) and springtails (WEISER & PURRINI, 1980) have already been described. Two new microsporidium parasites invading hemocytes of enchytraeids, two coccidians of nematodes, and a new gregarine of the family Monocystidae infecting *vesiculae seminales* of earthworms are to be published. Some of the important parasites are represented in Figures 1-23. They invaded different tissues : the gut, fat body, hemocytes, hypodermis, muscle fibres and reproductive organs. The invaded tissue totally degenerated in cases of heavy infections.

The complexity of the system of organisms inhabiting soil described above calls for more research to be done. The topics of future investigations could concentrate on the life cycle of microorganisms of soil invertebrates, their impact on their hosts and environment and effects of human activities responsible for their epidemic nature.

My very preliminary data on possible influence of environmental factors could be substantiated by future analyses of the influence of soil type, climate, industrial air pollution, SO_2 -immissions and other peculiarities.

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References

BERLIN (H.), 1924. — Untersuchungen über Monocystiden in der Vesiculae seminales der schwedischen Oligochaeten. — *Arch. Protistenk.*, **48** : 1-124.

BATHIA (B.L.), 1929. — On the distribution of Gregarines in Oligochaetes. — *Parasitology*, **21** : 120-131.

CANNING (E.U.), 1962. — *Legerella helminthorum* n.sp. a coccidian parasitic in a nematode. — *Arch. Protistenk.*, **105** : 455-462.

CANNING (E.U.), 1975. — *The Microsporidian Parasites of Platyhelmintes : their Morphology, Development, Transmission and Pathogenecety.* — CIH Miscellaneous publ. **2**, William Clowes & Sons Limited, London, 32 p.

COGNETTI DE MARTIIS (L.), 1911. — Contributo all conoscenza delle Monocystidae e dei loro fenomeni riproduttivi. — *Arch. Protistenk.*, **23** : 205-246.

DISSANAIKE (A.S.), 1957. — The morphology and life cycle of *Nosema helminthorum* Moniez. — *Parasitology*, **47** : 355-346.

DOLFFUS (R.P.), 1946. — *Parasites (animaux et végétaux) des Helminthes.* — Encyclopédie Biologique. Paris, Paul Lechevalier, 482 p.

ERHARDOVA (B.), 1955. — Prve nazely Gregarin v roztoku. — *Cesk. Parasitol.*, **2** : 35-37.

GEUS (A.), 1969. — Die Gregarinida der Land- und suesswasser bewohnenden Arthropoden Mitteleuropas. — *Tierw. Deutschlands*, **57** : 1-608.

HESSE (E.), 1909. — Contribution à l'étude des Monocystidées des Oligochètes. – *Arch. Zool. exp. et gén.*, **5** : 3-301.

KEILIN (D.), 1921. — On the life history of *Helicosporidium parasiticum* n.g. sp., a new type protist parasitic in the larvae of *Dasychelea obscura* Winn. (Diptera, Ceratopogonidae), and in some other Arthropods. — *Parasitology*, **13** : 97-113.

KOSTENKO (S.M.), AKIMOV (I.A.) & STARVIR (I.S.), 1978. — The occurrence of Gregarines in some acaroid tiks. — *Vestn. Zool.*, **2** : 61-64 (in Russian).

MEIER (M.), 1956. — Die Monocystiden-Fauna der Oligochaeten von Erlangen und Umgebung. — *Arch. Protistenk.*, **101** : 337-400.

MONIEZ (R.), 1879. — Note sur des parasites des helminthes. — *Bull. sci. Dept. Nord.*, **2** : 304.

PURRINI (K.) & BAEUMLER (W.), 1976. — *Nosema ptyctimae* n.sp. eine neue Mikrosporidie aus *Rhysotritia ardua* C.L. Koch (Euphtiracaridae, Oribatei). — *Anz. Schaedlingkde. Pflanzenschutz, Umweltschutz*, **49** : 169-171.

PURRINI (K.) & BAEUMLER (W.), 1977. — *Nosema hermaniae* n.sp. eine neue Mikrosporidie aus *Hermannia gibba* C.L. KOCH (Hermanniidae, Oribatei) in Fichtenwaldbestaenden. — *Zool. Anz.*, **1/2** : 107-112.

PURRINI (K.), BUVKA (V.) & BAEUMLER (W.), 1979. — Sporozoen in Hornmilden (Oribatei, Acarina) aus Walboden Sueddeutschlands nebst Beschreibung von *Gregarina postneri* n.sp. und *Gregarina fuscozoetis* n. sp. (Gregarinida, Sporozoa). — *Pedobiologia*, **18** : 329-339.

PURRINI (K.), 1980. — On the Incidence and Distribution of Parasites of Soil Fauna in Mixed Coniferous, Leaf, and Beech Forests of Lower Saxony, Federal Republic of Germany. *In* D.L. DINDAL (Ed.), *Soil biology as related to land use practices, proceedings of the VII Int. Colloquium of soil Zoology.* EPA (560/13-80-038), Washington : 561-582.

PURRINI (K.), 1981. — Studies on some Amoebae (Amoebida) and *Helicosporidium parasiticum* Keilin 1921 (Helicosporida) Infecting Moss-Mites (Oribatei, Acarina) in Forest Soil Samples. — *Arch. Protistenk.*, **124** : 303-311.

PURRINI (K.) & WEISER (J.), 1981. — Eight new microsporidian parasites of Moss-Mites (Oribatei, Acarina) in Forest soils. — *Z. ang. Ent.*, **91** : 217-224.

PURRINI (K.) & ORMIERES (R.), 1981. — Ueber vier neue Eugregarinene-Arten (Eugregarinida, Sporozoa) der Hornmilben (Oribatei, Acarina). — *Zool. Beitraege*, **27** : 123-132.

PURRINI (K.), 1982. — Light and Electron Microscopic Studies on Three Microsporidians (Microsporida : Phylum Microspora) Parasitizing Springtails (Collembola, Apterygota). — *Arch. Protistenk*, **126** : 385-394

PURRINI (K.), 1982. — Studies of Coccidian Parasites of Forest Soil Fauna — A Vacancy in Research Work as to Invertebrate Pathology. — (Unpublished).

PURRINI (K.), 1982. — *Vavraia collembolae* n.sp. a New Microsporidian (Microsporida : Phylum Microspora) Parasitizing Springtail *Onychiurus quadriocellatus* GISIN (Onychiuridae, Collembola). — (Unpublished).

RAY (N.H.), 1930. — Studies on some Sporozoa in Polychaete Worms. — *Parasitology*, **22** : 370-398.

STAMMER (J.H.), 1962. — Protozoen und Wuermer als Parasiten in Insekten. — *Deutsche Entomol. Zeitschr.*, **5** : 441-460.

THOR (S.), 1930. — Ueber einzellige Parasiten in verschiedenen Acarina. — *Z. Parasitenk.*, **2** : 551-570.

THORNE (G.), 1940. — *Dubosquia penetrans* n. sp. (Sporozoa, Microsporidia, Nosematidae), a parasitic of the nematode *Pratylenchus pratensis* (de Man) Filipijev. — *Proceedings of the Helminthological Society of Washington*, **7** : 5-53.

TROISI (R.L.), 1933. — Studies on the acephaline gregarines of some oligochaete annelids. — *Tr. Am. Micr. Soc.*, **52** : 326-352.

WEISER (J.) & PURRINI (K.), 1980. — Seven New Microsporidian Parasites of Springtails (Collembola) in Federal Republic of Germany. — *Z. Parasitenk.*, 62 : 75-84.

Illustrations

PLATE I

Figures 1-2 Mature spores of *Helicosporidium parasiticum* Keilin 1921, infecting fat body of oribatid-mite *Phthiracarus globosus.*

Figure 3 Body cavity (excepting the gut) of oribatid mite *Achipteria coleoptrata* filled with mature spores of *H. parasiticum.*

Figure 4 Developmental stages of a monocystid (Monocystidae, Gregarinidae) living in the gut lumen of Enchytraeidae sp.

Figure 5 Cyst with sporocysts of a monocystid (Monocystidae) living in the gut lumen of Enchytraeidae sp

Figure 6 Mature spores of microsporidian (*Nosema* sp.) infecting fat body of dipteran *Platosciarus socialis.*

Figure 7 Mature spores of microsporidian *Nosema enchytredidorum* n. sp. infecting hemocytes of enchytraeid *Fridericia ratzeli.*

Figure 8 Associated gamonts and solitary gamont of *Gregarina bisphaera* Purrini & Ormières (1981) living in the gut lumen of oribatid mite *Damaeus clavipes.*

(Light microscopy. — Figs 1 and 5 : Phase microscope; 2 and 3 : Heidenhain's iron haematoxylin staining; 4 and 8 : Unstained; 6 and 7 : Giemsa's stain).

PLATE II

Figures 9-16 Some developmental stages, oocysts and sporocysts in the life cycle of a new monocystid (Monocystidae, Gregarinida) infecting *vesiculae seminales* of earthworm *Lumbricus rubellus.*

Figures 9-11 Young non sporulated oocysts.

Figures 12,13 Maturing oocysts.

Figures 14,15 Mature oocysts containing macrosporocysts.

Figure 16 Mature oocyst containing microsporocysts.

(Light microscopy, unstained).

PLATE III

Figure 17 Mature oocysts containing sporocysts of coccidian *Adelina acarinae* sp. n. infecting fat body of oribatid mite *Nothrus silvestris.*

Figure 18 Ultrastructure of young sporocysts of coccidian *Adelina acarinae* sp. n.

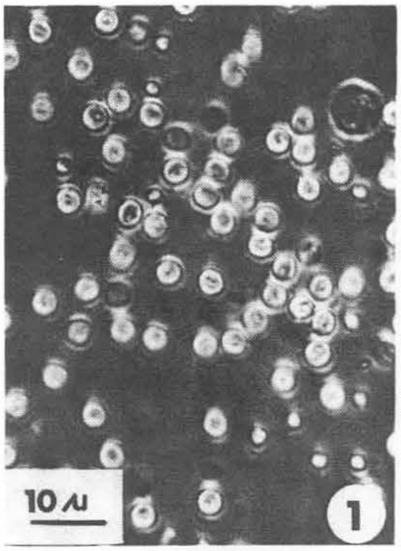
Figure 19 Ultrastructure of mature spore of a non-identified fungi parasitizing the gut of oribatid mite *Cepheus dentatus*, shows the spore wall (*SW*) composed of a thin electron dense layer (*a*) and a thick transparent layer (*b*), further shows the nucleus (*N*), vacuole (*V*), mitochondria (*M*) and some very electron dense plasma particles (*P*).

Figure 20 Bacteria (*Bacillus* sp.) parasitizing the gut of oribatid mite *Platynothrus peltifer.*

Figures 21,22 Ultrastructure of non identified microorganisms (virus like organisms) infecting fat body of oribatid mite *Damaeus clavipes.*

Figure. 22 Ultrastructure of mature spore of non identified microorganisms (Haplosporida-like protozoans, of fungi) infecting fat body of oribatid mite *Microtritia minima.*

(Light microscopy, Fig. 17 : unstained; Figs 18-23 : Electron Microscopy).



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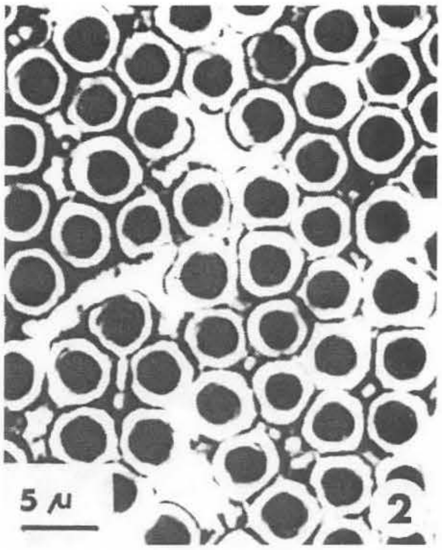

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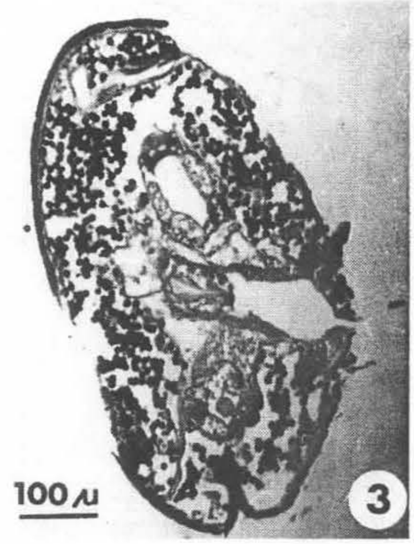

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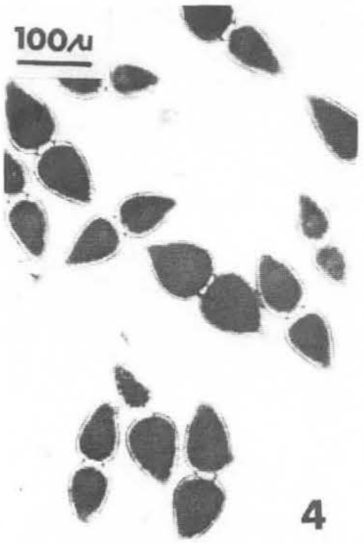

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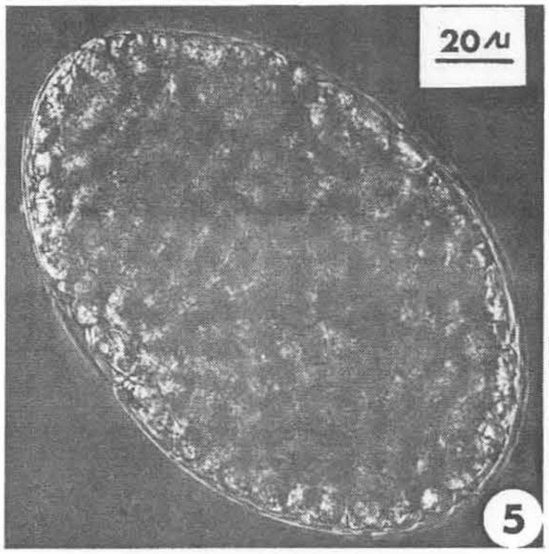

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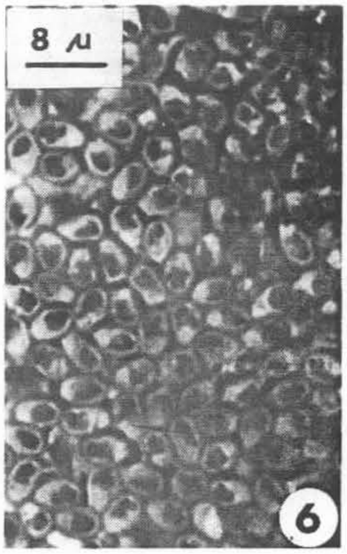

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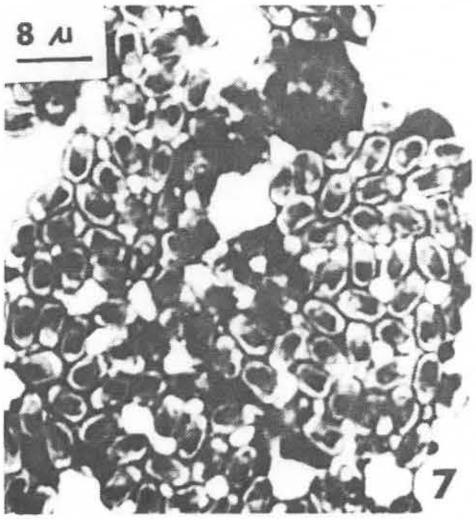

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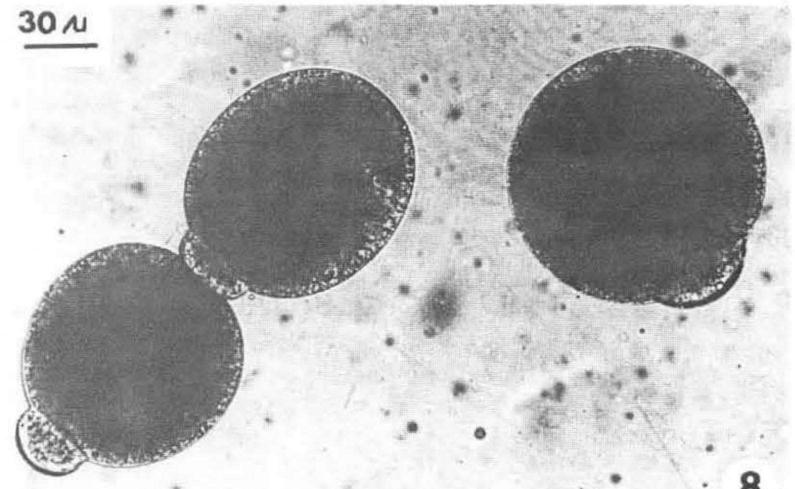

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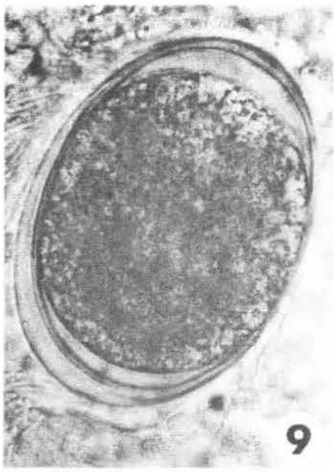
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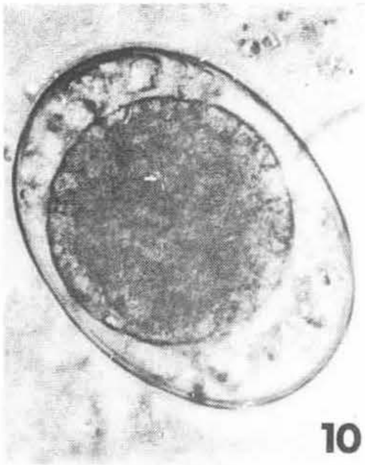
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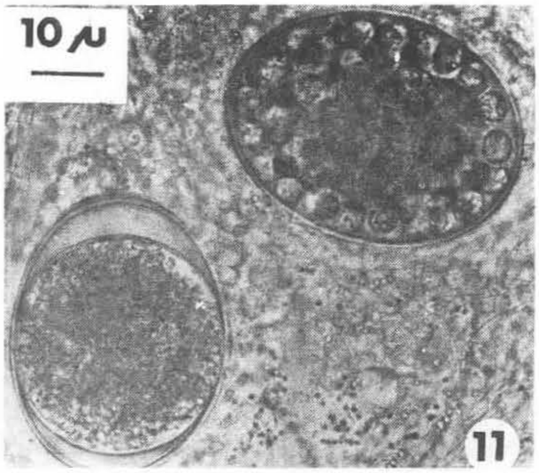
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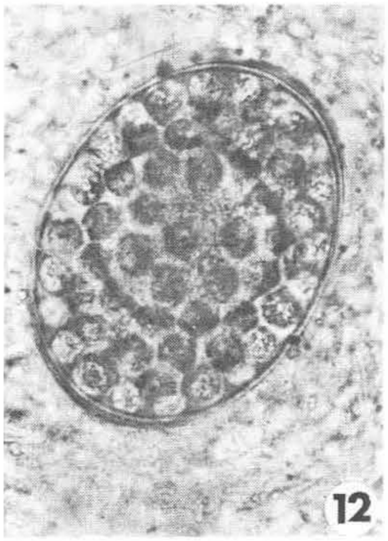
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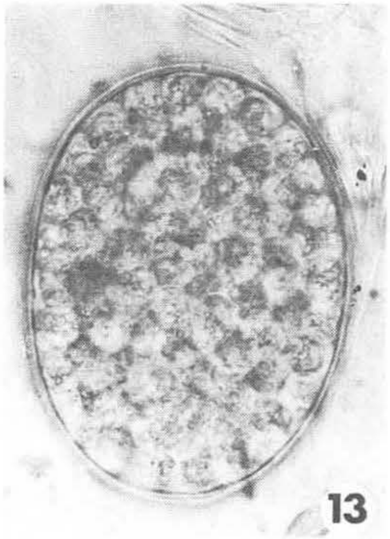
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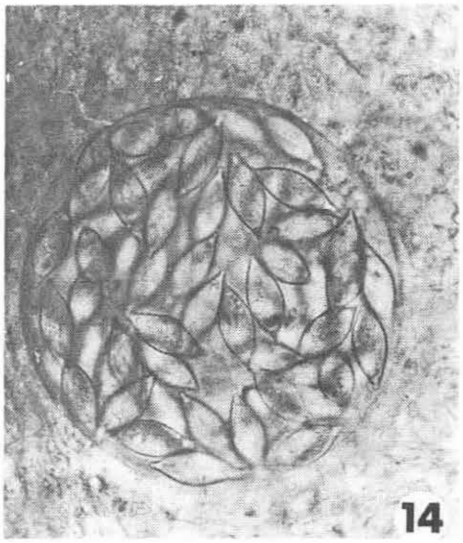
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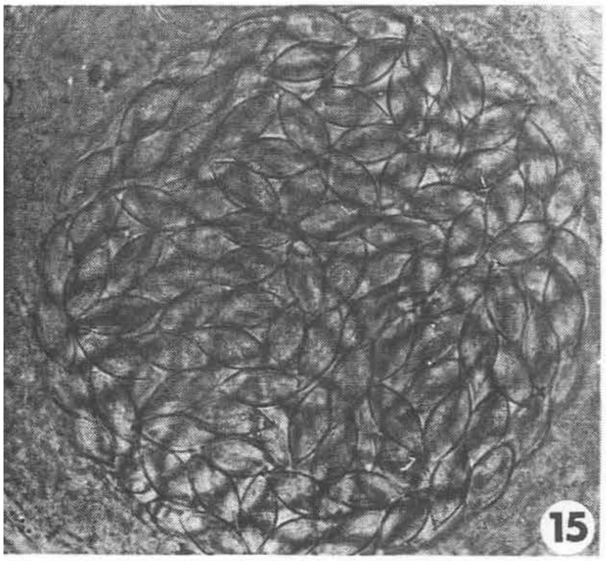
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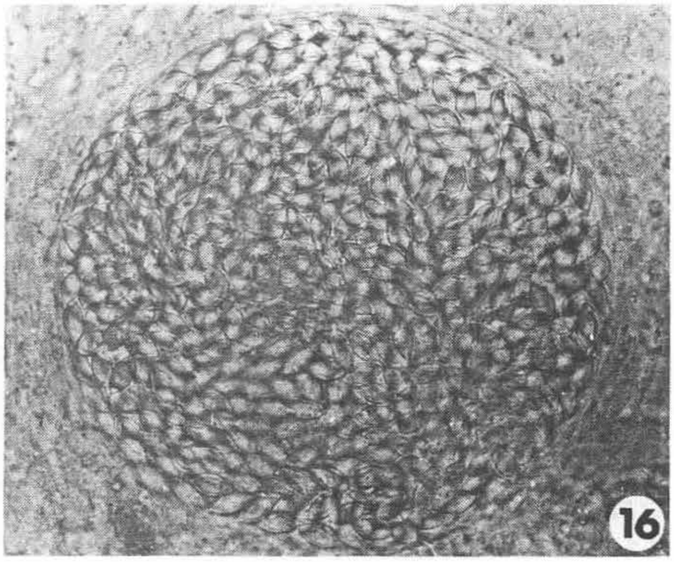
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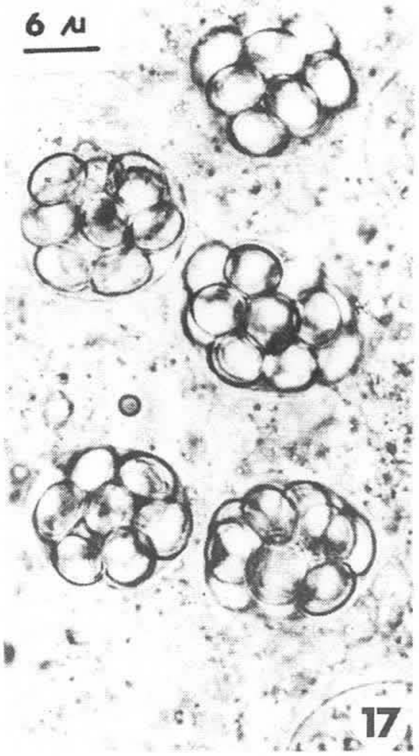

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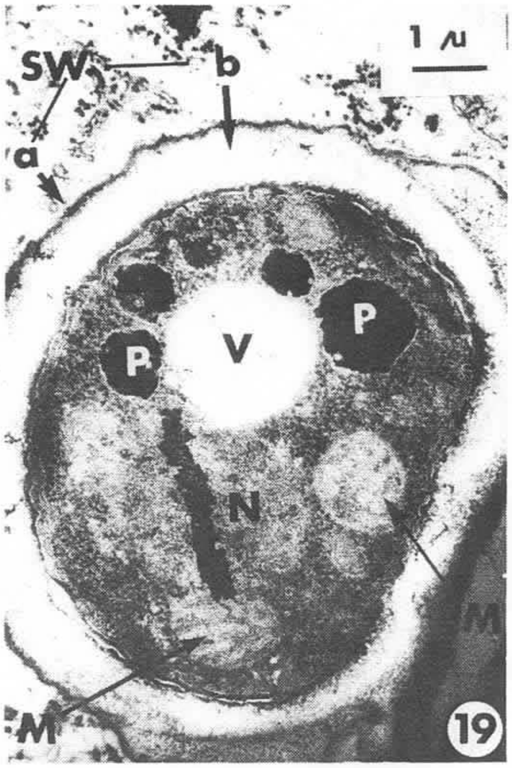

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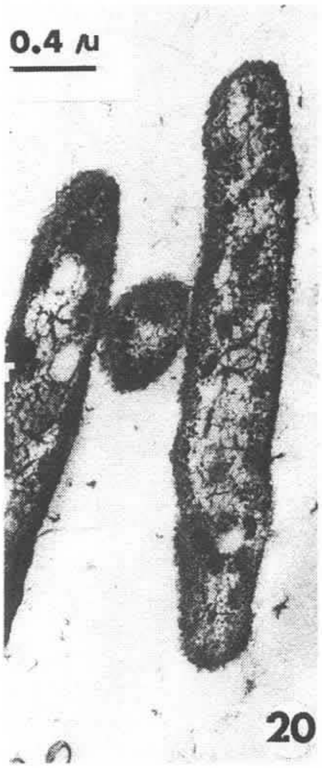

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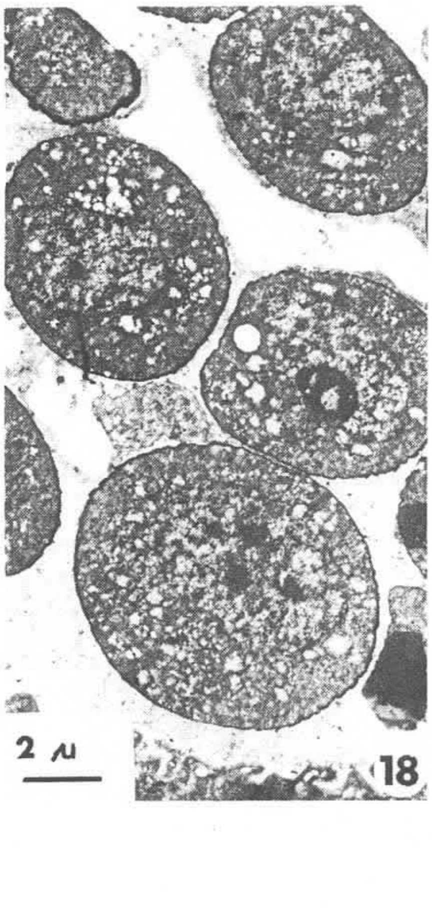

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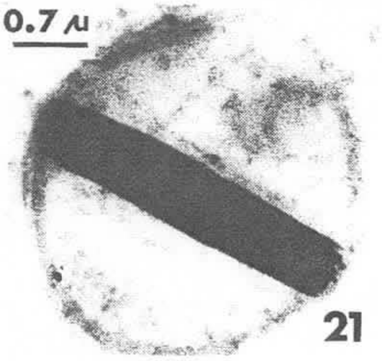

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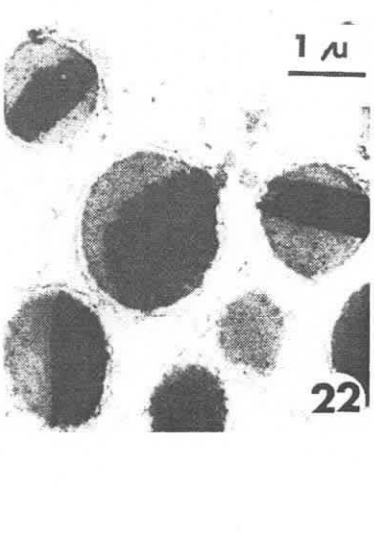

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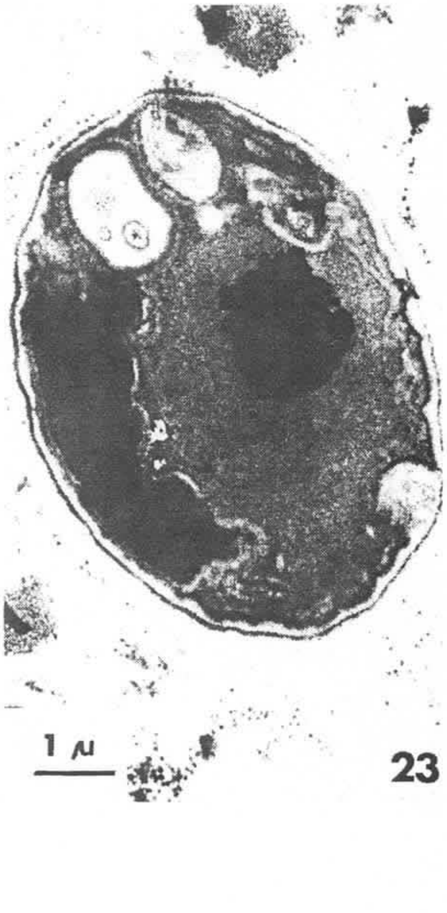

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